

Terrestrial Pulmonate Reproduction:
Seasonal and Annual Variation and Environmental Factors
in *Helminthoglypta arrosa* (Binney)

(Pulmonata : Helicidae)

BY

KENNETH L. VAN DER LAAN

Marine Science Institute, University of California, Santa Barbara, California 93106
and Bodega Marine Laboratory, University of California, Bodega Bay, California 94923

(1 Text figure)

INTRODUCTION

I REPORT HERE ON SEASONALITY in breeding, length of time between copulation and oviposition, length of time between oviposition and egg hatching, egg viability, and variation in reproductive rates in a population of the terrestrial pulmonate *Helminthoglypta arrosa* (Binney, 1855). I also consider the effects of water availability, food availability, and temperature on these aspects of reproduction. The snail population inhabited a lupine scrub stand (*Lupinus arboreus* Sims) on Mussel Point, Bodega Head, Sonoma County, California (38°20' N, 123°04' W), about 104 km north of San Francisco.

Helminthoglypta arrosa (1) occurs along the north-central California coast (PILSBRY, 1939), (2) aestivates during annual dry periods from mid-spring to early autumn (VAN DER LAAN, 1975b), and (3) terminates shell growth prior to reproductive maturity. The presence of a reflected shell lip indicates termination of growth: the adult phase. In four and one quarter years of field and laboratory observations (detailed below), snails with reflected shell lips were the only individuals that copulated and oviposited.

The sequence of breeding events in *Helminthoglypta* starts with copulation in the autumn at the end of long-term aestivation. Oviposition follows in the winter 40 to 90 days after copulation, and hatching occurs in early spring approximately 60 days after oviposition. I made no histological examinations but presume that in *Helmintho-*

glypta, as in other pulmonates (cf., DUNCAN, 1975), egg development precedes and embryogenesis follows oviposition. Duration of embryogenesis for *H. arrosa* thus was assumed to be the time between oviposition and hatching. On the other hand, in the absence of histological examinations, I made no assumption about the duration of ovarian development. This is because fertilization can occur before, or sometime after, copulation due to the fact that some pulmonates self-inseminate while others store sperm from another animal up to a year's duration (DUNCAN, 1975).

METHODS

Field study of copulation. I estimated seasonality and frequency of copulation in 1968 and 1969 for *Helminthoglypta* by walking through the lupine scrub stand (hereafter: "study area") and counting the number of adult snails and the number of snails copulating. I started each walk in late September prior to the time that copulation was first noted in 1966 and 1967. I continued the walks at least three weeks later than the last copulating snails were noted. Walks were conducted when snails were active: at night or during the day under conditions of overcast, fog, drizzle, or rain.

Laboratory study on temperature effects. I examined the relationships between temperature and duration of embryogenesis and between temperature and the probability

of hatching in laboratory experiments. Three egg masses of 63, 54, and 45 eggs were collected between 15 January and 4 March, 1969. I kept each third of each egg mass in the laboratory under constant temperatures of 4.4, 10.0, or 15.6° C. Periodically, hatched and unhatched eggs were counted. Hatchlings were removed upon discovery.

Regular field collections and observations. Variations in the densities of snails and snail eggs were used to estimate variations in reproductive output, in seasonality of oviposition and of egg hatching, and in duration of embryogenesis. I estimated egg and snail densities every 6 weeks or less between March, 1966, and December, 1969. These densities were determined by hand sorting through the vegetation and the upper 8 cm of soil at randomly selected quadrats. Neither snails nor eggs were found lower than 4 cm below the leaf litter during the study. Quadrats were usually 40 in number and 642 cm² (see VAN DER LAAN, 1971, for details).

I gained additional information on reproduction in *Helminthoglypta* from regular field observations which I made at least weekly and usually semiweekly from March, 1966, to June, 1970.

Environmental factors. Variations in rainfall, temperature, and food supply in the field were monitored. Preferences for particular plants as food were established in laboratory experiments and field observations (VAN DER LAAN, 1975a). The abundance of the preferred plants was estimated by recording the total length each plant intercepted along 1 m at each quadrat in regular collections taken November, 1967, to December, 1969.

Rainfall was collected in a standard rain gauge on Mussel Point and the amount recorded daily except for weekends. Ambient maximum and minimum temperatures 5 cm above the soil surface were recorded at least four times per week from November, 1967, to January, 1970.

Remarks on statistical treatment. Yates continuity correction was used for small sample sizes in the analyses of frequencies. Throughout I consider $p < 0.05$ to be significant. More exact probabilities, where calculated, are reported.

RESULTS

Season and Duration of the Mating Season

I observed *Helminthoglypta* copulating only in October and November and never at other times of the year in the

field (Table 1). In contrast, in the four and one quarter years of my study, one pair of laboratory-maintained snails copulated in March, 1966.

Table 1

Field observations of copulation: total number of adult snails observed and *in copulo* and percentage *in copulo* during the 1968 and 1969 mating seasons. For each year, no animals copulated prior to or after the dates shown below.

Date	Number of snails <i>in copulo</i>	Number of adult snails observed	Percentage <i>in copulo</i>
<i>1968</i>			
October 11	2	134	1.4
October 12	0	191	0.0
October 22	0	195	0.0
November 1	0	222	0.0
November 2	0	246	0.0
November 5	2	199	1.0
Σ	4	1187	
<i>1969</i>			
October 8	14	217	6.4
October 10	0	190	0.0
October 14	2	232	0.9
November 2	5	201	2.5
November 3	4	210	1.9
November 7	0	140	0.0
November 15	2	214	0.9
November 26	2	164	1.2
Σ	29	1568	

For the two years of my study I first noted copulating pairs on 11 October, 1968, and on 8 October in 1969. Onset of breeding was coincident with reactivation from long-term aestivation and occurred within 24 hours of the first October rainfall that thoroughly wetted the vegetation and leaf litter. In contrast to this circumstance is that, after heavy rains in August and September of 1968, snails emerging from aestivation did not copulate but reentered aestivation. I observed the last snails copulating on 5 November for 1968 and 26 November for 1969. I saw no snail *in copulo* in subsequent semi-weekly field observations, which I continued into spring, nor in more intense 15 min. searches, which I continued until mid-January and which totaled 1272 snails and six and three-quarters

hrs. in 1968 and 90 snails and one and three-quarters hrs. in 1969.

Frequency of Copulation

In every instance, except one, I observed mutual copulation in *Helminthoglypta*. The mean percentage of adults *in copulo* was greater in 1969 compared to 1968 (Table 1). In both years the highest proportion of snails *in copulo* was observed within the first 24 hrs. following onset of the mating seasons (11 October, 1968; 8 October, 1969 (Table 1). The highest percentage of snails copulating 8 October, 1969, was significantly greater than for 11 October, 1968 ($X^2 = 5.02$, d.f. = 1, $p < 0.05$, n for 1968 = 140 snails, n for 1969 = 217).

The density of food at the onset of the breeding season in 1969, which was the year of higher copulation frequencies, was greater than in 1968; however, this difference was not significant (1.6% cover of food plants on 10 October, 1968 and 6.5% on 8 October, 1969, $X^2 = 1.96$, d.f. = 1, $p > 0.10$).

Cumulative monthly rainfall in September and October, 1969 (38.1 and 105.6 mm), was also greater than in 1968 (5.1 and 53.3 mm) and higher, although not significantly so, than mean monthly rainfall for 1967 to 1977 (14.3 and 49.0 mm). Rainfall for November, the end of the mating season, on the other hand, was lower in 1969 (48.3 mm) than in 1968 (203.0 mm).

Helminthoglypta was active at field temperatures above 4° C and *in copulo* only at ambient temperatures of 10 to 15° C at night or during the days when it was overcast, foggy, drizzling, or raining. When snails were active, they were just as likely to copulate during the day as during the night. Copulation rates during the night were not significantly different from rates during the day (Table 2).

I also noted no significant correlations (Pearsons product-moment coefficient) of copulation frequencies with maximum or minimum temperature in the 24 hrs. previous to the observation walks I took during the breeding seasons.

Variations in Reproductive Output

During my regular field collections I found 37 egg masses. Oviposition sites and sizes of eggs were in exact agreement with previously reported observations (INGRAM, 1947) for *Helminthoglypta arrosa* occurring on Point Reyes, approximately 22 km southeast of Mussel Point. In my study all egg masses were in shallow holes in the soil

Table 2

Percentage of adult snails *in copulo* during daylight and nighttime (n = total number of adult snails observed during the breeding season).

	Daylight	Nighttime
<i>1968</i> - $\chi^2 = 0.01$, d.f. = 1, $p > 0.90$		
Percent	0.35	0.32
n	571	616
<i>1969</i> - $\chi^2 = 2.43$, d.f. = 1, $p > 0.90$		
Percent	2.50	1.40
n	639	929
<i>1968 and 1969. Combined</i> - $\chi^2 = 1.50$, d.f. = 1, $p > 0.10$		
Percent	1.48	0.97
n	1218	1545

below the leaf litter. The mean diameter of eggs was 2.2 mm (n = 104, standard deviation = 0.2 mm). As in *Helminthoglypta tudiculata* (INGRAM & ADOLPH, 1942), eggs at oviposition had an elastic translucent outer membrane. Later the membrane became opaque, white and slightly stiffer. The mean number of eggs per egg mass was 75.6 (range 45-171, standard deviation = 30.0). The number of eggs per egg mass did not vary significantly (Model I ANOVA, $F = 0.24$, d.f. = 2, 31) among the 1967, 1968, and 1969 egg laying seasons. However the density of eggs/m² and hence the number of eggs was significantly greater ($X^2 = 52.1$, d.f. = 2, $p < 0.001$) in 1969 than in 1967 and 1968; hence, fecundity was highest in 1969. Variation in egg densities between years was likely a result of greater egg production per adult snail since densities of adults were not significantly different among years for the months (November to March) when presumably eggs were oviposited ($X^2 = 2.84$, d.f. = 2, n.s.). There was, moreover, a negative nonsignificant correlation ($r = -0.590$, n = 14) between egg and adult densities. Variation in rainfall, however, appears to be important in egg production. Egg density was positively correlated ($r = 0.977$, n = 3, $p < 0.05$) with total rainfall between December and March, the peak season of egg appearance (see below and Figure 1).

Seasonality of Egg Appearance and Egg Hatching

High densities of eggs occurred in my regular field collections starting in late November and ending in early

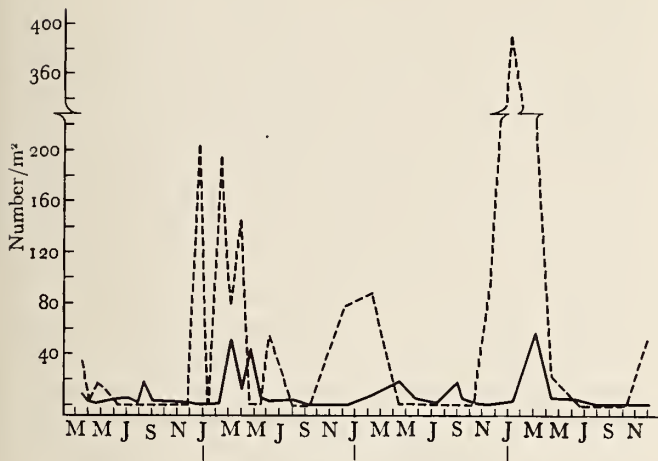


Figure 1

Density (number/m²) of eggs (----) and hatchlings (—) on Mussel Point from March, 1966, to December, 1969. Inflexion points represent actual collection dates

April. Viable eggs were found in the field as late as June. Peak numbers of eggs were evident between late December and early March (Figure 1).

I collected newly hatched snails (recognized by their small size, their lack of shell growth, and the hirsute condition of their shells) in peak numbers in March and April and as early as February and as late as August (Figure 1). I also found young-of-year in late summer that were small, had no shell growth, and were hirsute. These snails prob-

ably hatched in late spring rather than in summer since they were all in aestivation when collected. Aestivation for the whole population, moreover, starts in mid- to late spring (VAN DER LAAN, 1975b).

Length of Time between Copulation and Oviposition, Duration of Embryogenesis, and Probability of Hatching

Results from the laboratory study indicated that *Helminthoglypta* eggs are likely to be fertile if oviposited: 94% of all eggs held at the highest temperature hatched. I also found that with higher temperatures, eggs hatched sooner and more successfully (Table 3).

In the field the modal length of time between copulation and egg hatching for *Helminthoglypta* was 6 months: between early October, when copulation frequencies were high, and March and April, when peak hatching occurred. The maximum possible development time was 8 months: the time between the first copulation and the latest hatchings (early June) and the minimum possible time was 2½ months: the time between the end of the copulation season (late November) and the first hatchings (mid-February).

Recall that I was not able to estimate the duration of ovarian development. Since snails could have stored sperm or self-inseminated, fertilization could have occurred prior to or sometime after copulation. I note, however, that the time between copulation and oviposition can be as short as 42 days; in 1968 copulation was first observed on 11 October and eggs were first collected on 22 November.

Based on the difference in time between the start of the peaks in numbers of eggs and of hatchlings (Figure 1) and assuming that embryogenesis starts with oviposition, I

Table 3

Cumulative percentage of eggs hatching kept in the laboratory at three temperatures for three egg masses, collected in the field winter 1969. N = number of eggs in each egg mass. Days = days held at each temperature.

Temperature (°C)	Collected 15 January N = 45				Collected 11 February N = 63				Collected 4 March N = 54					
	11	16	19	74	5	11	19	27	74	5	11	19	27	74
4.4	0	0	0	0	0	0	0	0	0	0	5	83	83	83
10.0	53	73	73	73	0	0	0	0	0	78	100	—	—	—
15.5	67	93	93	93	0	0	71	90	90	100	—	—	—	—

estimated the modal duration of embryogenesis for *Helminthoglypta* to be 2 months.

DISCUSSION

Most reproductive events in *Helminthoglypta arrosa* occur during the annual wet season. The exceptions are: sperm development, which must take place prior to copulation, and ovarian development, which may also occur earlier than mating. Copulation is coincident with the start of the wet season, and hatching is completed by the start of the dry season. Food availability, water availability, and temperature have direct and indirect and proximate and ultimate effects on reproduction. These external factors also interact with each other. Food availability depends on water availability. Higher primary productivity occurs in the wetter years, and, as in other Mediterranean climates, plant phenological events depend on rainfall patterns. In addition, times of lower temperatures are coincident with times of higher rainfall (VAN DER LAAN, 1971).

For *Helminthoglypta arrosa* rainfall appeared to be the only variable that explained differences in copulation rates between years of my study. Neither food availability nor temperature was significantly different between years.

For some snails and slugs temperature plays a role in determining the seasonality of mating (DUNCAN, 1975). In many other terrestrial pulmonates rainfall and water availability are the principal correlates of seasonality (DUNCAN, *op. cit.*; HEATWOLE & HEATWOLE, 1978). Timing of the onset of the breeding season for *Helminthoglypta arrosa* may depend on both food and water availability, but probably is not dependent on temperature variations. Relatively low autumn temperatures slow soil desiccation, and temperatures below 4°C, which occurred only twice during the two breeding seasons, resulted in snail inactivity. Otherwise snails were active under the full range of temperatures that occurred during the breeding seasons. Food may be an ultimate factor in initiation of the mating season. The abundance of food plants rapidly increased from early to late autumn (a two- to ten-fold increase in abundance during my study). Water availability is both a proximate and ultimate factor in initiating the mating seasons. Rainfall in early October results in annual reactivation from long-term aestivation (VAN DER LAAN, 1975b) by thoroughly wetting the leaf litter and soil. Thus, rainfall probably is the signal for the onset of copulation. High water availability is absolutely necessary for snail activity and facilitates snail feeding, acting thereby as an ultimate factor.

The mating season could have ended simply because every potentially reproductive animal was inseminated or every animal used up an annual sperm supply, or both. By assuming that *Helminthoglypta* remained in copulo 6.5 hrs., as did *Helix aspersa* (Müller) (HERZBERG & HERZBERG, 1962) and by assuming that snails were active at night and during days that had fog, overcast, rain, or drizzle, I found that a projection of copulation frequencies indicated that 100% of the *Helminthoglypta arrosa* population would have copulated by mid-November in 1969; my observation was that copulation ceased at the end of November, 1969. In 1968, a year of low copulation frequencies, only 50% of the population would have copulated by late November. Copulation, however, ended at the start of November. Furthermore, copulation ended by late November in 1966, 1967, and 1970. Thus, variation in environmental factors rather than sperm supply is strongly implicated in terminating the mating season.

Late autumn variation in rainfall, food availability, and temperature did not appear to be proximate factors in the cessation of the breeding season, but springtime patterns in water availability and in food availability were ultimate factors in terminating the mating season. Copulation later than the end of November could result in very high mortality for eggs and newly hatched snails on Mussel Point. Given a development period of 6 months, copulation that occurred later than November would result in unhatched eggs or newly hatched snails in mid-spring or later. Such eggs and hatchlings would probably die at very high rates. *Helminthoglypta* eggs, like slug eggs (RUNHAM & HUNTER, 1970), have no known specializations to prevent water loss. In fact, most eggs I collected in late spring were quite brittle or even cracked open. Newly hatched snails in late spring face a declining food supply (I recorded a drop in food plant abundance from 50% to 12% cover in March to May, 1969) and a substantial reduction in water availability occurs each spring (mean monthly rainfall for 1967 to 1979 was 45.7 mm for April, 8.5 mm for May, and 3.7 mm for June). This cohort also experiences very high mortality during the annual dry periods (VAN DER LAAN, 1975b). I note that late autumn cessation of copulation as an adaptation to springtime patterns in water and food may be peculiar to the Mussel Point population. *Helminthoglypta arrosa* was observed in copulo in February on Pt. Reyes, 22 km southeast of my study area (INGRAM, 1947).

I postulate that egg size and moisture availability affect the duration of embryogenesis in terrestrial gastropods. Furthermore, even though I could not determine the age of eggs when they were collected, it appears that there is

an inverse relationship between duration of embryogenesis and temperature (Table 3). This is consistent with the results obtained by CARRICK (1942) for *Agriolimax agrestis* Linnaeus.

Given this inverse relationship, duration of embryogenesis for *Helminthoglypta arrosa* in the field (mode = 2 months) is probably lengthened by the cool temperatures on Mussel Point (minimum temperatures ranged between 2.5 and 7.5° C for December to April in the two years of my study, while maximum temperatures were 15.8 to 17.4° C). I note that embryogenesis in the field took one and one-half months in Puerto Rican tree snails (HEATWOLE & HEATWOLE, 1978) and 30 to 45 days in the African giant snail (AJAYI *et al.*, 1978). Egg size (suggested here as a correlated variable) was not reported for the African snail. Puerto Rican snails, however, had eggs about 4 times larger than *Helminthoglypta* eggs.

I compared three other aspects of reproduction in *Helminthoglypta arrosa* to other pulmonates. First, mean clutch size (75.6 eggs/mass) was higher than that for most similarly sized helicids (11-96 eggs/clutch) and approximated the median clutch size for terrestrial pulmonates in general (HYMAN, 1967). Secondly, the hatching rate that I recorded for *H. arrosa* (94%) is close to the 91.3% rate for *Achatina fulica* Bowdich from Hawaiian populations held at room temperature (KEKAUOHA, 1966). *Agriolimax agrestis*, however, had no egg mortality at 5° C and 37% mortality at 20° C (CARRICK, 1942). Finally, the total presence of mutual copulation observed for *Helminthoglypta arrosa* is in contrast to INGRAM & ADOLPH'S (1942) observations of frequent one-way copulations in laboratory-maintained *Helminthoglypta tudiculata*, but is in keeping with HYMAN'S (1967) conclusion that pulmonates generally practice reciprocal insemination. Mutual mating may, in fact, always occur in *H. arrosa*, since mutual copulation could have occurred just after or just before my single brief observation of one-way copulation.

SUMMARY

1. Seasonality in mating, length of time between copulation and oviposition, duration of embryogenesis, egg viability, and variation in egg production were studied in a northcentral California coastal population of the land snail *Helminthoglypta arrosa* (Binney, 1855). The effects of water availability, food availability, and temperature on these aspects of reproduction are discussed.

2. Copulation was reciprocal in every instance except one observation of short duration.
3. Copulation only occurred in October and November, began after reactivation from long-term aestivation, was coincident with heavy and frequent rainfall that thoroughly wetted the leaf litter and soil, and was coincident with a rapid increase in abundance of food plants.
4. It is argued that the timing of the cessation of the mating season is adapted to the spring decline in water and food availability that hatchlings experience.
5. Copulation rates were higher in 1969 than in 1968 when rainfall was significantly higher and food abundance was higher, although not significantly so.
6. Variation in copulation rates during the mating season was not significantly different between daytime and nighttime and was not significantly correlated with variation in either maximum or minimum temperatures.
7. Eggs averaged 2.2 mm in diameter ($n = 105$, s.d. = 0.2 mm). The mean numbers of eggs/egg mass (75.6) were not significantly different among three years; however, the density of eggs (number/m²) was significantly higher for one year. Variation in egg density was positively correlated with rainfall. Furthermore, changes in population egg production depended on changes in reproductive output/adult since adult densities did not vary among years.
8. In laboratory experiments eggs held at 15.6, 10.0, and 4.4° C hatched sooner and more successfully the higher the temperature.
9. In the field the modal length of time between copulation and hatching was 6 months (range 2½ to 8 months) with a modal duration of 2 months for embryogenesis. Duration of embryogenesis was probably lengthened by low field temperatures.

ACKNOWLEDGMENTS

I wish to thank Alice Kingsbury, Armand Kuris, José Javier Alió, Eric Hochberg and an anonymous reviewer for helpful suggestions on the manuscript. Data were collected at the Bodega Marine Laboratory (B.M.L.), Uni-

versity of California (U.C.), Bodega Bay, while holding a pre-doctoral fellowship No. 5 Fo1 GM3643-03 from the National Institute of Health at the Department of Zoology, U.C., Berkeley. Data were analyzed while sponsored by NSF Grant No. OCE 78-08489 at the Marine Sciences Institute, U.C., Santa Barbara. The Director, Cadet Hand, and the staff at B.M.L. provided important logistic support.

Literature Cited

- AJAYI, S. S., O. O. TEWE, C. MORIARTY & M. O. AWESU
1978. Observations on the biology and nutritive value of the African giant snail, *Archachatina marginata*. E. Afr. Wildl. Journ. 16 (2): 85-95; 2 text figs. (June 1978)
- CARRICK, ROBERT
1942. The grey field slug, *Agriolimax agrestis* L. and its environment. Ann. Appl. Biol. 29: 43-55; 4 text figs.
- DUNCAN, CHRISTOPHER J.
1975. Reproduction. In: Vera Fretter & J. Peake, eds., Pulmonates. Acad. Press, London, New York, xxix+417 pp.; illust.
- HEATWOLE, HAROLD & AUDRY HEATWOLE
1978. Ecology of Puerto Rican camaenid tree-snails. Malacologia 17 (2): 241-315; 40 text figs.
- HERZBERG, FRED & ANNE HERZBERG
1962. Observations on reproduction in *Helix aspersa*. Amer. Midl. Nat. 68 (2): 297-306; 5 text figs. (October 1962)
- HYMAN, LIBBIE HENRIETTA
1967. The Invertebrates. 6, Mollusca 1: vii+792 pp.; illust. McGraw-Hill Book Co., N. Y., St. Louis, London
- INGRAM, WILLIAM MARCUS
1947. A contribution to the natural history of *Helminthoglypta arrosa* ('Gld.' Binney) and *Helminthoglypta nickliniana awania* Bartsch. Bull. So. Calif. Acad. Sci. 46 (2): 81-83 (May-August 1947)
- INGRAM, WILLIAM MARCUS & HELEN M. ADOLPH
1942. Life history data on *Helminthoglypta tudiculata*. Bull. So. Calif. Acad. Sci. 41 (2): 97-101 (May-August 1942)
- KEKAUOHA, WILLARD
1966. Life history and population studies of *Achatina fulica*. The Nautilus 80 (1): 3-10 (July 1966) and 80 (2): 39-46 (October 1966); 2 text figs.
- PILSBRY, HENRY AUGUSTUS
1939. Land Mollusca of North America (north of Mexico). Acad. Nat. Sci. Philadelphia Monogr. 3; 1 (1): i-xvii, 1-573, i-ix; text figs. 1-377 (6 December 1939)
- RUNHAM, N. W. & P. J. HUNTER
1970. Terrestrial slugs. Hutchinson Univ. Libr. London 1-184; 8 pls.; 5 text figs.
- VAN DER LAAN, KENNETH LEROY
1971. The population ecology of the terrestrial snail, *Helminthoglypta arrosa* (Pulmonata: Helicidae). vii+235 pp.; illust. Ph. D. thesis, Univ. Calif. Berkeley, California
- 1975a. Feeding preferences in a population of the land snail *Helminthoglypta arrosa* (Binney) (Pulmonata: Helicidae). The Veliger 17 (4): 354-359 (1 April 1975)
- 1975b. Aestivation in the land snail *Helminthoglypta arrosa* (Binney) (Pulmonata: Helicidae). The Veliger 17 (4): 360-368; 1 text fig. (1 April 1975)

